

Multiple-Quantum NMR Studies of Monomeric Bonded Silica Phases[†]

Covalently modified, bonded silica phases have been characterized by proton multiple-quantum (MQ) nuclear magnetic resonance (NMR) spectrometry. In the case of trimethylsilyl bonded phases, the loading has been shown to be homogeneous in that MQ coherence development plateaus at a value of approximately 10 correlated protons. Thus, at room temperature the MQ-NMR spin "counting" result is consistent with the fact that trimethylsilyl groups have 9 protons. The groups are dispersed such that intermolecular dipolar interactions are minimal, and the protons of individual trimethyl groups are behaving collectively as isolated clusters. Similar results are presented for a C₈ monomeric phase (at -101 °C) where isolated clusters of approximately 25 protons have been detected, and 23 are seen on the individual groups of the C₈ monomeric phase. The mobility of the alkyl chain has been shown to vary depending upon the structure of the immobilized ligand. At room temperature the mobility of the C₈ alkyl chain groups reduces the dipolar interaction among its protons. Therefore, MQ-NMR was run at the lower temperature to limit molecular motion while enhancing proton dipolar coupling.

Introduction

Most high-performance liquid chromatography separations are currently carried out in the reversed-phase mode. Novel bonded phases have been developed for the separation of specific classes of compounds, and new substrates have been introduced which characteristically permit usage over wide pH ranges with extended column lifetimes.¹ Solute retention in liquid chromatography mechanisms, however, are not fully understood. The physical and chemical characteristics of the bonded phase are directly associated with such mechanisms.

Monomeric alkyl phases are produced by reactions of monofunctional silanes (e.g., monochloro- or monoalkoxysilanes) with silanols as shown schematically in Figure 1.¹ Monomeric phases can also be produced by the reaction of silica with di- or tri-

functional silanes. Water, however, must be excluded from such reactions to preclude silane polymerization. Reaction of a monofunctional silane with silica results in the formation of a single-bond linkage for each silane molecule that reacts with the silica.

The extent of surface modification is dependent upon the reaction conditions as well as the physical, geometric, and chemical properties of the substrate and silane reagents. Steric constraints can restrict the number of surface groups which can be chemically modified. Ligands which are already attached to the silica surfaces may shield neighboring unbonded silanol groups from reaction. The homogeneity of such loading is controversial and is the subject of considerable research.^{2,3} In this paper we examine the dispersion of groups which have been covalently linked to the silanol surface. Are the groups uniformly distributed, or do such mod-

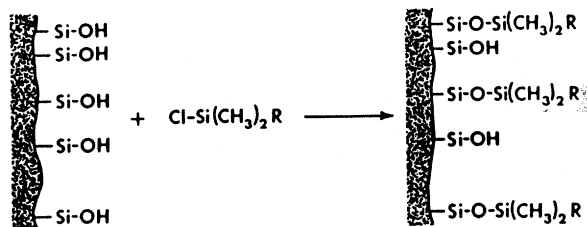


Figure 1. Monomeric bonded phase synthesis using monofunctional silanes.

ifications of the surfaces with alkyl ligands produce phases of concentrated surface coverage and other areas of low coverage? Classical characterization methods and calculations involving bulk properties provide an average measure of phase surface coverage. However, such approaches are not very specific in answering questions with respect to molecular level ligand dispersion or clustering.

In this report we demonstrate that solid-state ^1H multiple-quantum (MQ) NMR, together with spin counting, can reveal the spatial distribution of nuclei in materials (such as the bonded phases presented here) which lack long-range order.⁴⁻⁹ Time reversal pulse sequences have made MQ investigation of large spin systems in solids a reality.^{4,10-11}

We present the first study of the application of MQ-NMR to two covalently modified, bonded silica phases. The structures of these phases are depicted in Figure 2. The data presented are related to the distribution of the ligands on the substrates which were studied. The size and extent of hydrogen clustering are related to the density and proximity of the neighboring alkyl ligands, the strength of their intermolecular dipolar interactions, and the development (or lack of development) of multiple quantum spin coherence among these entities. Furthermore, the development of multiple-quantum coherence within such systems (or the correlation of the ^1H spins within the molecule) has been analyzed on the basis that the pattern and rate of growth of such correlations reflect the distribution of atoms within the system.

Experimental Section

Vydac silica was purchased from Separations Group, Hespera, CA.¹⁸ The silica gel was deuterated by exchange in D_2O prior to derivatization with the silyl-alkyl ligands. The C1 phase was prepared by adding 10 mL of trimethylchlorosilane to 2.02 g of the deuterated silica. 4-(Dimethylamino)pyridine (1 g) was added as a catalyst.¹² The mixture was subsequently filtered and dried under vacuum. The C8 phase was prepared by adding 10 g of octyldimethylchlorosilane to the deuterated silica along with 100 mL of CCl_4 . The mixture was allowed to stand at room temperature for 4 h and subsequently filtered and dried. All of the NMR spectra were acquired on a home-built multiple quantum spectrometer operating at a frequency of 100 MHz for the proton resonance. The proton single-quantum spectra were obtained by the standard, single-pulse Fourier transform method. Phase-incremented multiple-quantum spectra were obtained with the pulse sequence shown in Figure 3. The experimental parameters employed were $\Delta = 2.0 \mu\text{s}$, $\Delta' = 8.0 \mu\text{s}$, fixed $t_1 = 2 \mu\text{s}$, pulse width

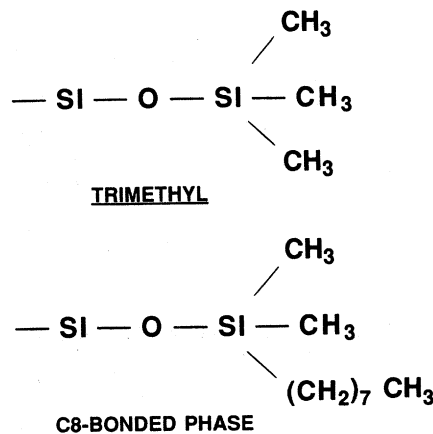


Figure 2. Silyl derivatives examined by MQ NMR: (A) trimethylsilyl bonded phase (C1 bonded phase); (B) C8 monomeric bonded phase.

$t_{90} = 4.0 \mu\text{s}$, and $1 \leq \tau/\tau_c \leq 20$. The basic cycle time τ_c was $72 \mu\text{s}$. Detection was by pulses spin locking for 1 ms after the mixing period. The sequence was phase-cycled to remove the undesirable (for our purposes) odd-order MQ coherences by cycling the pulse phases during the preparation period between 0° and 180° in alternate experiments and co-adding the resulting signals. The phase-incremented multiple-quantum sequence has been discussed elsewhere in detail.¹¹

Results and Discussion

The multiple-quantum NMR technique enables one to circumvent the Zeeman selection rule $\Delta M = 1$. Such multiple-quantum coherence can be created among groups of spins through the application of appropriate radio frequency (rf) pulse sequences. The spins can then interact collectively as a result of their dipolar couplings.^{4,13} Under the sequence used here,⁸ a system of $N^{1/2}$ spins in a solid will evolve dynamically under the following dipolar Hamiltonian:

$$H = -\frac{1}{2} \sum_{i < j} D_{ij} (I_i^+ I_j^- + I_i^- I_j^+) \quad (1)$$

I_+ and I_- are the usual spin angular momentum raising and lowering operators. D_{ij} is the dipolar coupling constant between two spins i and j . This Hamiltonian will eventually result in a coherent superposition of states with differences in magnetic quantum number of $0, \pm 2, \pm 4, \dots, \pm N$. For the spins to become correlated with one another, the Hamiltonian must act for some time τ , which is proportional to the inverse of the dipolar interaction. As the coupling constant is inversely proportional to the cube of the interproton distance, nearby spins interact over short time periods, whereas spins which are farther apart require relatively more time to communicate. If a solid contains isolated clusters, the dipolar couplings among groups may be negligible, and an upper limit may be imposed on the size of the spin system or on the average number of interacting nuclei. Thus clusters of protons can be treated as independent spin systems with an upper bound on the maximum number of spins which can interact. Initially and over short time periods therefore, the development of MQ coherence is induced and varies as a function of the preparation time. In a cluster, however, the number of correlated spins will eventually become constant with time, and the effective system size detected in the MQ experiments will reflect the size of the isolated group of nuclear spins. If groups of clusters exist near to one another or if the system is comprised of a continuous distribution of spins, the effective system size will continually increase over (preparation) time. Therefore, a series of MQ experiments of varying preparation times can be used to determine the effective system size and whether nuclei are clustered or homogeneously distributed.^{13,14}

(4) Baum, J.; Munowitz, M.; Garraway, A. N.; Pines, A. *J. Chem. Phys.* **1985**, *83*, 2015.

(5) Drobny, G.; Pines, A.; Sinton, S.; Weitekamp, D. P.; Wemmer, D. *Faraday Symp. Chem. Soc.* **1979**, *13*, 49.

(6) Bodenhausen, G.; Vold, R. L.; Vold, R. R. *J. Magn. Reson.* **1980**, *37*, 93.

(7) Pines, A. In *Proceedings of the Fermi School on the Physics of NMR in Biology and Medicine*; Maraviglia, B., Ed.; in press.

(8) Garraway, A. N.; Baum, J.; Munowitz, M. A.; Pines, A. *J. Magn. Reson.* **1984**, *60*, 337.

(9) Ryoo, R.; Liu, S.-B.; de Menorval, L. C.; Takegashi, K.; Chmelka, B.; Trecocke, M.; Pines, A. *J. Phys. Chem.* **1987**, *91*, 6575.

(10) Yen, Y. S.; Pines, A. *J. Chem. Phys.* **1983**, *78*, 3579.

(11) Shykind, D. N.; Baum, J.; Liu, S.-B.; Pines, A.; Garraway, A. N. *J. Magn. Reson.* **1988**, *76*, 149.

(12) Sentell, K. B.; Barnes, K. W.; Dorsey, J. G. *J. Chromatogr.* **1988**, *455*, 95-104.

(13) Gerasimowicz, W. V.; Garraway, A. N.; Miller, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 3726-3730.

(14) Weitekamp, D. P. *Advances in Magnetic Resonance*; Waugh, J. S., Ed.; Academic Press: New York, 1990; Vol. 11, pp 111-274.

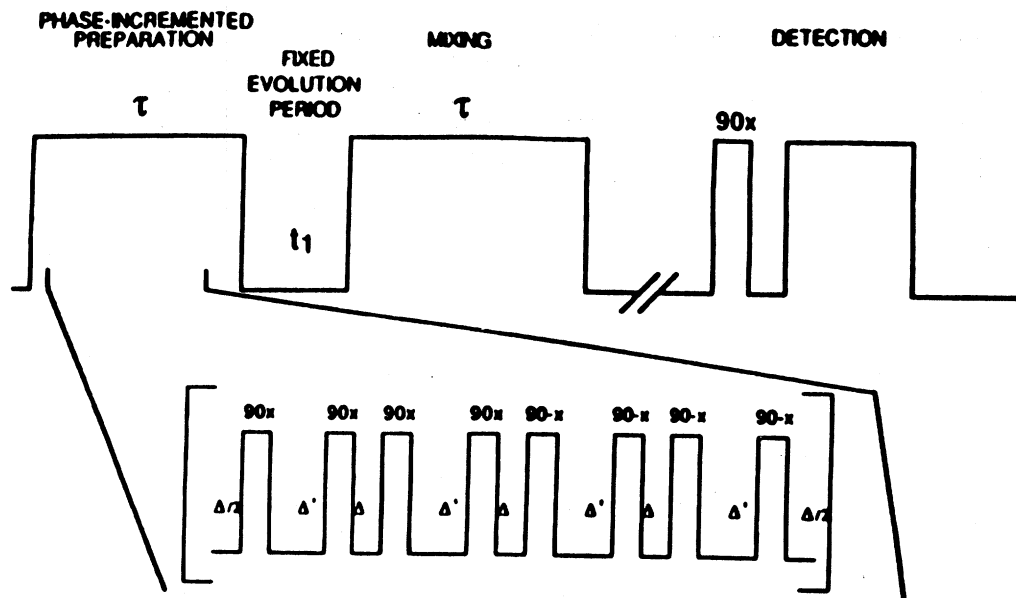


Figure 3. MQ NMR pulse sequence: $\Delta = 2.0 \mu\text{s}$, $\Delta' = 8.0 \mu\text{s}$, fixed $t_1 = 2.0 \mu\text{s}$, pulse width $t_{90} = 4.0 \mu\text{s}$, and $1 \leq \tau/\tau_{90} \leq 20$. The phase of the 90° rf pulse is indicated by x or -x. The basic cycle time τ_c was $72 \mu\text{s}$. Detection was by pulsed spin locking.

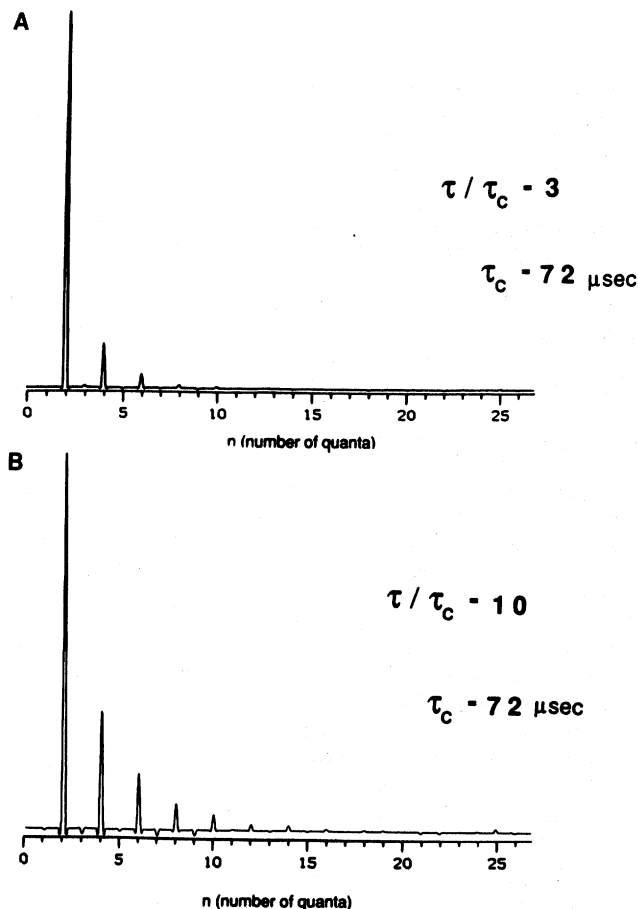


Figure 4. (A) Phase-incremented multiple-quantum NMR spectra of the trimethylsilyl bonded phase where $\tau/\tau_c = 3$ and the basic cycle time is $72 \mu\text{s}$. (B) Same as in part A except $\tau/\tau_c = 10$.

Representative multiple-quantum NMR spectra of the trimethylsilyl bonded phase for two different preparation times ($\tau/\tau_c = 3, 10$) are shown in Figure 4 (room temperature). These spectra indicate that higher MQ orders are clearly seen at the longer preparation time. At the longer preparation time, some very minor (insignificant) intensities are seen beyond $\Delta M = 20$ in Figure 4B. These signals are due to amplitude modulation of the pulses as the cycle proceeds and do not affect the calculations or results discussed below. The silica gels were deuterated prior to deriv-

atization with the trimethyl group. Presumably, the protons contributing to the MQ signal are strictly from these groups. A nonderivatized but deuterated silica gel was examined under these same MQ conditions, and no evidence of coherence development was detected.

For a spin system of $N^{1/2}$ particles, the number of transitions between coherent states differing by $n = \Delta M$ is given by

$$\binom{2N}{N - \Delta M} = 2N! / [(N - \Delta M)!(N + \Delta M)!] \quad (2)$$

The multiple-quantum intensity distribution, $\mathcal{J}(n)$, can be related to the number of multiple-quantum transitions within a particular order. This distribution may be described by a Gaussian curve¹⁵⁻¹⁷

$$\mathcal{J}(n) = \exp(-n^2/N) \quad (3)$$

with a variance of

$$\sigma = (N/2)^{1/2} \quad (4)$$

The integrated intensities of the MQ transitions are fit to a Gaussian where the σ of the Gaussian is associated with $(N(\tau)/2)^{1/2}$. Such a fit corresponds to the approximation that at any preparation time τ , the distribution of coherence within the spin system is reasonably represented as an equilibrium distribution within a fictitious spin system of size $N(\tau)$. Figure 5 shows a plot of the number of correlated proton spins versus τ/τ_c for the trimethylsilyl derivative. Beyond a value of $\tau/\tau_c \approx 10$, a plateau is reached. This behavior points to a constant value for the overall system size. No further increase in the effective system size is evident beyond some limit characteristic of the apparent cluster size, in this case approximately 10 ± 1 spins.

The asymptotic maximum of about 10 correlated proton spins is consistent with the picture that on average, the trimethylsilyl groups of 9 protons are well-separated from their nearest neighbors. Our result resolves the situation of distribution of these ligands in favor of homogeneous dispersion rather than concentrated pockets. Chemical analysis of these materials indicates that there are 1.35 groups/nm^2 . The chemical analysis, however,

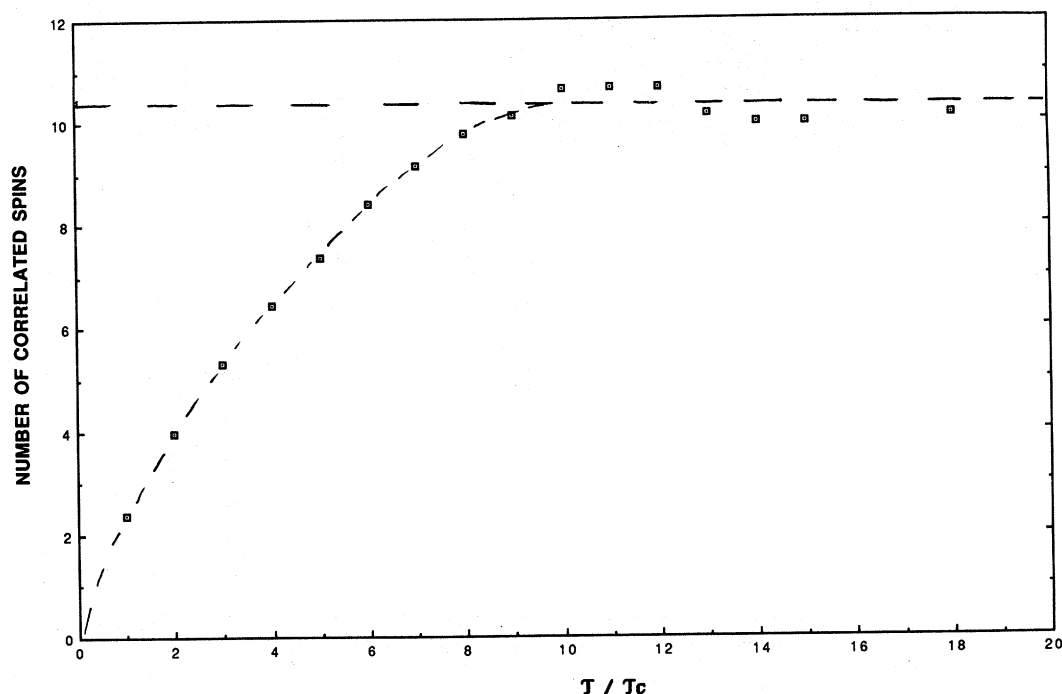


Figure 5. Number of correlated spins versus preparation time (τ) for the trimethylsilyl derivative.

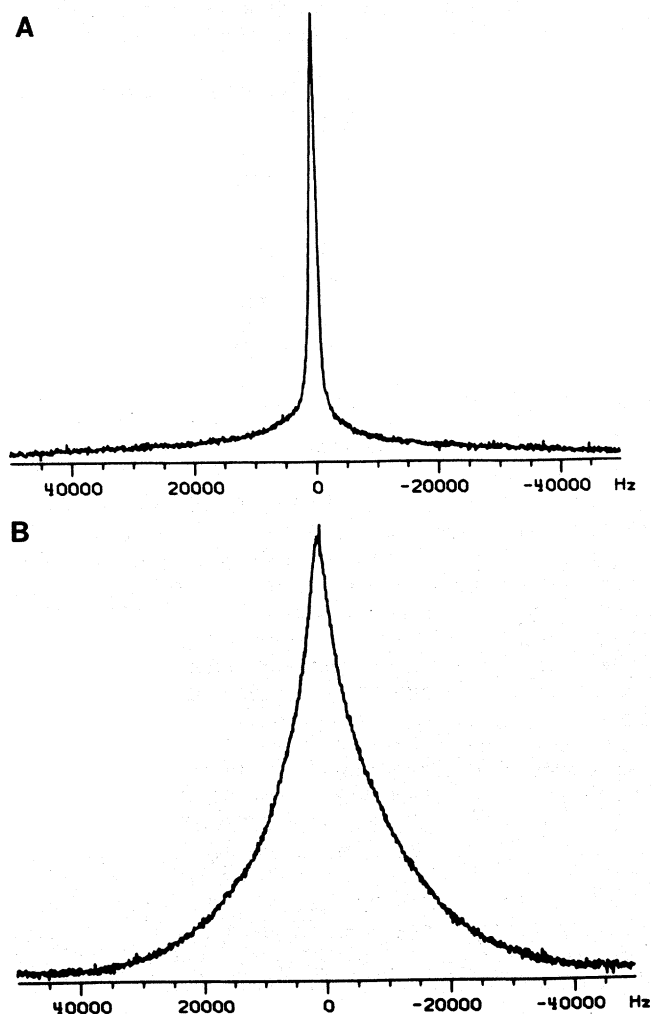


Figure 6. Single-quantum ^1H NMR spectra of the C8 monomeric bonded phase: (A) room-temperature spectrum; (B) spectrum taken at -101°C .

provides a value only for average coverage. It does not specify the internuclear separations of the groups. On the other hand, MQ NMR provides a real measure of the statistical distribution of the actual derivatized sites.

In the case of the C_8 monomeric phase, the multiple-quantum NMR experiment was not feasible at room temperature. Figure 6 depicts single-quantum proton spectra of this derivatized surface at both room temperature and at -101°C . At room temperature the solid-state ^1H resonance line (Figure 6A) is narrowed. Such narrowing in a solid is indicative of molecular motion which modulates the intra- and intermolecular proton dipolar interactions. Therefore, a lack of dipolar coupling due to alkyl group motion limits the applicability of the MQ NMR experiment at room temperature for this system.

At -101°C , the line width is much broader (Figure 6B), indicating that the motion of the alkyl group(s) has been restricted considerably, and the MQ experiment was now carried out at this lower temperature. The effective system size was found to approach a limit or constant value of approximately 25 spins. Chemical analysis of these materials is consistent with the MQ result and indicates that coverage levels (on average) are less than 1.35 groups/ nm^2 . Each functional group on the C_8 bonded phase contains 23 protons. Thus, for this case the MQ experiment also resolves the distribution of the alkyl groups into a pattern of homogeneously distributed clusters which correspond to the individual organic functional species.

Conclusion

In conclusion, the combination of ^1H spin counting and multiple-quantum NMR experiments provides a representation of the manner in which bonded phase materials have been loaded. These methods can be directly related to the characterization of the extent and effectiveness of surface modification in this important class of substances. Finally, the MQ technique has been demonstrated to be applicable to measurements of amorphous surface and lattice coverage, derivatization, and saturation phenomena.

Acknowledgment. This work was supported in part by the Office of Naval Research.